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Growth inhibition of human cancer cells in vitro by T-type calcium channel blockers

Jae Yeol Lee,[a,*] Seong Jun Park,[b,c] Sung Jun Park,[a] Min Joo Lee,[a] Hyewhon Rhim,[c] Seon Hee Seo[c] and Ki-Sun Kim[d]

[a]*Research Institute for Basic Sciences and Department of Chemistry, College of Sciences, Kyung Hee University, 1 Hoegi-Dong, Seoul 130-701, Republic of Korea*
[b]*Department of Chemistry, Korea University, 1-Anamdong, Seoul 136-701, Republic of Korea*
[c]*Life Sciences Division, Korea Institute of Science & Technology, PO Box 131, Cheongryang, Seoul 130-650, Republic of Korea*
[d]*Department of Chemical Engineering, Daebul University, Young Am, Chonnam 526-702, Republic of Korea*

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Abstract—This paper describes the preliminary biological results that novel T-type calcium channel blockers inhibit the growth of human cancer cells by blocking calcium influx into the cell, based on unknown mechanism on the cell cycle responsible for cellular proliferation. Among the selected compounds from compound library, compound **9c** (**KYS05041**) was identified to be nearly equipotent with Cisplatin against some human cancers in the micromolar range.

Calcium plays a critical role as an intracellular signal, and controls many different cell processes,[1] of which calcium appears to play an important role in cell growth.[2] For example, it has also been shown that calcium signaling is required for cell cycle progression from G1/S phase through mitosis.[3–6] It has been demonstrated that depletion of intracellular calcium arrests the cell cycle in the G0/G1 and S interphases.[7] Regulation of the changes in intracellular calcium has been proposed to be via a T-type calcium channel.[8] Lined with this proposition, it has recently been reported that T-type calcium channel blockers (CCBs) inhibited cellular proliferation.[9,10] Opposed to T-type CCBs, however, it remains to discussion that some L-type CCB as anti-hypertensive agents may be related to the risk of cancer in the elderly and promote growth of pre-existing cancer cells in human by inhibition of apoptosis.[11] Therefore, selective T-type CCBs could be another tool to treat cancer where the cell cycle has become aberrant. Based on these findings, we also decided to evaluate the inhibitory activity of 3,4-dihydroquinazoline derivatives as novel T-type calcium channel blockers against the growth of human cancer cells in order to find another relationship between T-type channel blocking and the growth inhibition of cancer cells.

By using the synthetic methods according to Scheme 1, we have recently prepared a library of 3,4-dihydroquinazoline derivatives and evaluated their channel blocking effects against T-type channel (α_{1G}) expressed on HEX293 cell by whole cell patch-clamp methods: T-type Ca^{2+} currents were evoked every 15 s by a 50 ms depolarizing voltage step from −100 to −30 mV and percentage inhibition was calculated as $100[(C - D)/C]$, where D is the peak current in the presence of drug, and C is the peak current before the application of drug.[12] As a result, we have disclosed a few of lead compounds (**KYS05064** and **KYS05071**) as novel T-type channel blockers showing a good potency and channel selectivity through intensive SAR study.[13] From the compound library, we have selected eight compounds showing a broad range of channel blocking effects (34–91% inhibition at 10 μM concentration) to find their relationship between channel blocking effect and growth inhibition of cancer cells, and the structures of selected compounds are shown in Figure 1.

The selected compounds were evaluated for their growth inhibition against five cancer cell lines, including human

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* Corresponding author. Tel.: +82 2 9610726; fax: +82 2 9663701; e-mail: ljy@khu.ac.kr

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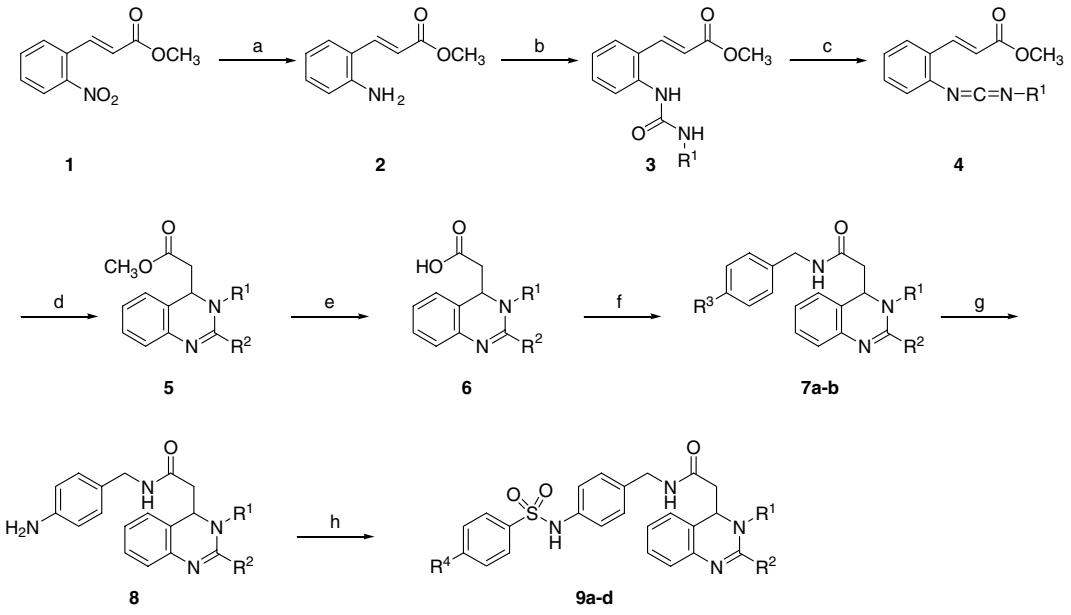

Scheme 1. Reagents and conditions: (a) $SnCl_2{\cdot}2H_2O$, EtOAc, 70 °C; (b) R^1NCO, benzene, rt; (c) $Ph_3P{\cdot}Br_2$, Et_3N, CH_2Cl_2, 0 °C; (d) R^2H, THF, rt; (e) $LiOH{\cdot}H_2O$, THF–H_2O (1:1), 60 °C; (f) *p*-R^3–$BnNH_2$, HOBT, EDC, rt; (g) 10% Pd(C), MeOH, rt; (h) *p*-R^4–$PhSO_2Cl$, pyridine, 0 °C to rt.

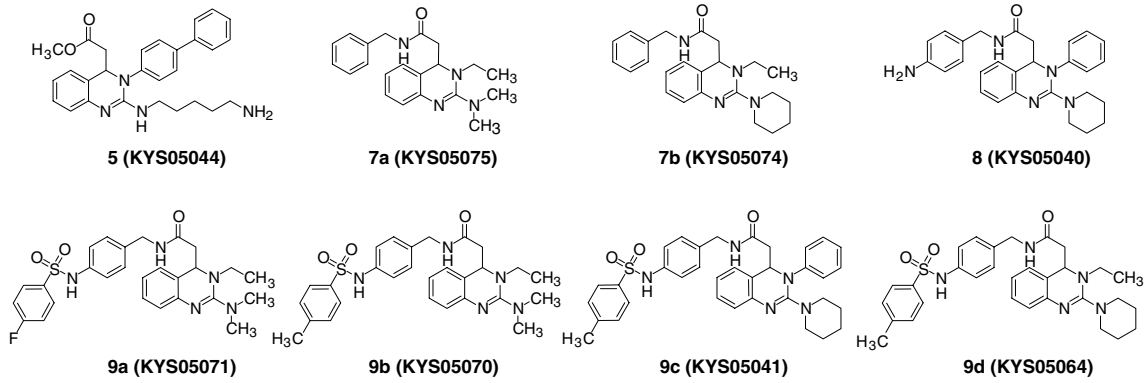

Figure 1. 3,4-Dihydroquinazoline derivatives studied in SRB assay.

lung carcinoma (A-549), human colon cancer (HCT-15), human epidermoid carcinoma (KB), human malignant melanoma (SK-MEL-2) and human ovarian cancer (SKOV3) using sulforhodamine B (SRB) assay:[14] All cell lines were grown in RPMI 1640 (Gibco BRL) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS) and maintained at 37 °C in a humidified atmosphere with 5% CO_2. The cells were seeded into 96-well plate. Various concentrations of samples were added to each well in triplicate and then incubated at 37 °C with 5% CO_2 for 72 h such that cells are in the exponential phase of growth at the time of drug addition. After incubation, 100 μL of formalin solution was gently added to the wells. Microplates were left for 30 min at room temperature, washed five times with tap water. One hundred microliters of 0.4% SRB solution was added to each well and left at room temperature for 30 min. SRB was removed and the plates were washed five times with 1% acetic acid before air-drying. Bound SRB was solubilized with 100 μL of 10 mM unbuffered Tris-base solution (Sigma) and plates were left on a plate shaker for at least 10 min. The optical density was measured using a microplate reader (Versamax, Molecular Devices) with a 520 nm wavelength and the growth inhibition concentration was expressed as GI_{50}. The results of assay are summarized in Table 1 as well as the channel blocking effect (% inhibition values at 10 μM concentration). Cisplatin was used as a reference for anti-cancer activity and also tabulated with data for comparison. In addition, blocking effects against N-type channel are also inserted into Table 1 in order to investigate the N-type channel blocking effect on growth inhibition of cancer cells.

Before discussing the results, we have already confirmed that all of the selected compounds did not show any cytotoxicity on HEX293 cell at 100 μM concentration as reported previously.[13] From these data, a brief relationship profile emerged as follows: as expected, Cisplatin barely blocked T-type Ca^{2+} currents and the mean percentage of its inhibition is 3.9 ± 2.1 ($n = 3$) at 10 μM concentration of Cisplatin, which acts by

Table 1. Biological data for selected compounds

Compound	Channel blocking effect (% inhibition)[a,b]		Growth inhibition of cancer cell: GI_{50} (μM)[c]				
	T-type (α_{1G})	N-type (α_{1B})	A-549[d]	HCT-15[e]	KB[f]	SK-MEL-2[g]	SKOV3[h]
5 (KYS05044)	82.5 ± 0.7	No blocking	3.39 ± 0.11	3.64 ± 0.24	3.77 ± 0.07	3.32 ± 0.20	3.24 ± 0.31
7a (KYS05075)	34.4 ± 1.4	27.6 ± 0.5	>200	>200	>200	50.19 ± 2.45	>200
7b (KYS05074)	59.3 ± 1.9	9.3 ± 0.5	95.10 ± 19.97	74.16 ± 9.63	108.14 ± 24.43	42.48 ± 0.00	77.84 ± 12.62
8 (KYS05040)	43.5 ± 4.5	8.9 ± 1.8	36.60 ± 1.19	33.86 ± 3.86	37.08 ± 0.82	30.73 ± 5.97	39.32 ± 1.28
9a (KYS05071)	62.7 ± 2.3	No blocking	66.10 ± 19.10	38.62 ± 3.13	41.63 ± 7.41	31.36 ± 1.53	46.48 ± 5.29
9b (KYS05070)	81.3 ± 0.8	8.9 ± 1.8	39.16 ± 6.97	33.89 ± 2.75	33.83 ± 0.56	24.21 ± 5.08	33.44 ± 0.00
9c (KYS05041)	89.9 ± 1.3	14.1 ± 2.1	3.06 ± 0.10	2.80 ± 0.28	3.11 ± 0.25	2.70 ± 0.21	2.93 ± 0.00
9d ((KYS05064)	91.3 ± 0.6	No blocking	23.03 ± 0.75	25.67 ± 8.61	22.28 ± 1.80	3.52 ± 0.18	22.78 ± 5.15
Cisplatin	3.9 ± 2.1	ND[i]	0.67 ± 0.67	2.80 ± 1.00	1.50 ± 1.20	4.40 ± 1.40	1.80 ± 0.67

[a] % Inhibition on HEX293 cell at 10 μM.
[b] Value was determined from dose–response curve and obtained from three independent experiments.
[c] GI_{50} value was determined from dose–response curve and obtained from three independent experiments.
[d] Human lung carcinoma (A-549).
[e] Human colon cancer (HCT-15).
[f] Human epidermoid carcinoma (KB).
[g] Human malignant melanoma (SK-MEL-2).
[h] Human ovarian cancer (SKOV3).
[i] ND, not determined.

crosslinking DNA in several different ways, making it impossible for rapidly dividing cells to duplicate their DNA for mitosis. In general, all compounds showed the similar correlation between cell growth inhibitory activity and T-type channel blocking effect, even though it is non-linear. That is, compound **7a** showing weak activity against T-type channel produced weak activity against cancer cells. Compounds **5** and **9c** showing strong potency against T-type channel exhibited strong activity against cancer cells. All compounds also exhibited the similar growth inhibitory activities against five cancer cells except for compounds **7a**, **7b**, and **9d**. Interestingly, compound **9d**, which is the most active compound (91.3 ± 0.6%) against T-type channel, exhibited less activity than compounds **5** and **9c** except for SKMEL2 cell line. This result could be explained by comparing IC_{50} values of compounds **5**, **9c**, and **9d**, which are 0.56 ± 0.10, 0.17 ± 0.08, and 0.96 ± 0.22 μM, respectively (the data not inserted in Table 1). With respect to IC_{50} value, therefore, the most active compound **9c** exhibited the meaningful inhibition of cancer cell's growth compared to Cisplatin, which is our reference compound.

As shown in Table 1, it seems that the blocking of N-type channel has no effect on the anti-cancer activity. That is, the sum of T-type and N-type % inhibition did not contribute to increase the growth inhibition activity, when considered the data of two pairs of compounds such as **7a** and **8**, and **7b** and **9a**. For the exact relationship between N-type channel blocking and anti-cancer activity, however, compounds showing the selectivity for N-type channel should be synthesized and evaluated for anti-cancer activity.

In conclusion, we have provided the additional evidence for the relationship between the inhibition of calcium influx and anti-cancer activity via T-type calcium channel blocking even though it is only preliminary data. This result is obviously found to coincide with the previous works by other research groups and also endows T-type calcium channel blocker with the possibility as another anti-cancer agent.[15,16] However, more work is needed in order to understand the calcium influx and cell proliferation via T-type calcium channel. Encouraged by these results, therefore, the synthesis and biological evaluation of a variety of compounds are in progress for providing the direct linkage and will be announced in the future.

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